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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND DACLATASVIR IN DRUG PRODUCT BY RP-HPLC

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ABSTRACT
The aim of the method was to develop and validate a rapid, sensitive and accurate method for simultaneous estimation of Daclatasvir and Sofosbuvir in drug product by liquid chromatography. The chromatographic separation was achieved on Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) at ambient temperature.The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (50:50). The flow rate was 1.0ml/ minute and ultraviolet detector at 275nm. The average retention time for Daclatasvir and Sofosbuvir found to be 2.805 min and 3.734 min. The proposed method was validated for selectivity, precision, linearity, and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 12.0 -36.0µg/mL for Daclatasvir and 80.0 -240.0µg/mL of Sofosbuvir.

KEYWORDS: Daclatasvir, Sofosbuvir, Isocratic, HPLC, Phenyl, Trifluoroacetic acid, Acetonitrile, Methanol, and validation.

1. INTRODUCTION

Daclatasvir

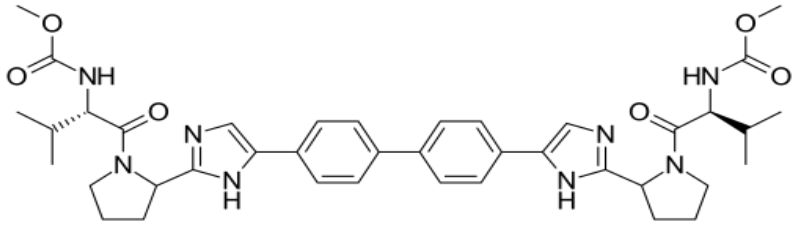


Fig. 1: Chemical structure: Daclatasvir.

Daclatasvir, sold under the trade name **Daklinza**, is a medication used in combination with other medications to treat hepatitis C (HCV). The other medications used in combination include sofosbuvir, ribavirin, and interferon, vary depending on the virus type and whether the person has cirrhosis. It is taken by mouth once a day.

Daclatasvir is chemically designated as Dimethyl *N*,*N*'-([1,1'-biphenyl]-4,4'-diylbis{1*H*-imidazole-5,2-diyl-[(2*S*)-pyrrolidine-2,1-diyl][(2*S*)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate. Its molecular formula is $C_{40}H_{50}N_8O_6$, and its molecular weight is 738.89 g/mol.

Sofosbuvir

Sofosbuvir, sold under the brand name **Sovaldi** among others, is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon- alfa, simeprevir, ledipasvir, daclatasvir, or velpatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved.Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby. It is taken by mouth.

Sofosbuvir is chemically designated as Isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate. Its molecular

formula is C24H29N5O3, and its molecular weight is 529.453 g/mol.

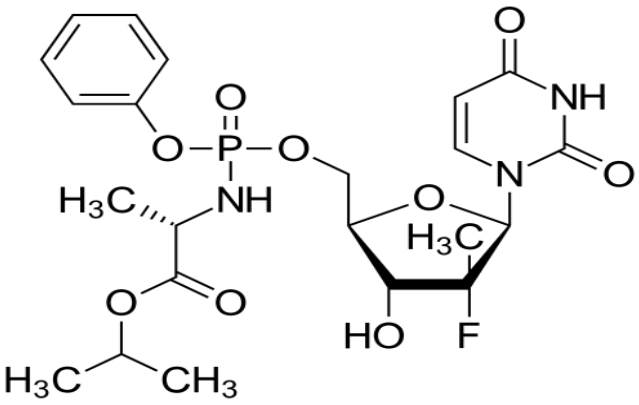


Fig. 2: Chemical structure: Sofosbuvir.

2. MATERIALS AND METHODS

2.1 Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5µm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance and Vacuum microfiltration unit with a 0.45µ membrane filter.

2.2 Materials: Pharmaceutically pure sample of Daclatasvir/Sofosbuvir were obtained as gift samples from Fortune pharma training institute, Sri Sai Nagar Colony, KPHB, Hyderabad, India.

HPLC-grade Methanol and Acetonitrile were obtained from qualigens reagents Pvt Ltd. Trifluoroacetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions: The sample separation was achieved on an (Eclipse XDB-Phenyl 250*4.6, 5µm) Phenyl column, aided by mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (50:50). The flow rate was 1.0 ml/ minute and ultraviolet detector at 275nm that was filtered and degassed prior to use, Injection volume is 10µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation: Taken accurately 1ml of Trifluoroacetic acid in 1000mL of water Mobile phase: Then added 50 volumes of buffer and 50 volumes of Acetonitrile mixed well and sonicated for 5 min.

Diluents: Water: Acetonitrile: 50:50 v\v.

2.4 Preparation of solutions

2.4.1 Standard solution: 6mg of pure Daclatasvir and 40 mg of Sofosbuvir were weighed and transferred to 50 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 2ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 24.0µg/ml of Daclatasvir and 160.0µg/ml Sofosbuvir.

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 6mg of Daclatasvir and 40mg of Sofosbuvir sample and transferred to 50 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution, 2 ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 24.0µg/ml of Daclatasvir and 160.0µg/ml Sofosbuvir.

2.5 Method validation

2.5.1. System suitability

The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system.

2.5.2. Linearity

Linearity was studied by analyzing five standard solutions covering the range of 12.0 -36.0µg/ml for Daclatasvir and 80.0 -240.0µg/ml Sofosbuvir. From the primary stock solution 1ml, 1.5ml, 2ml, 2.5ml, 3 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 12.0 µg/mL, 18.0µg/mL, 24.0µg/mL, 30.0µg/mL and 36.0µg/mL of Daclatasvir and 80.0g/mL, 120.0µg/mL, 160.0µg/mL, 200.0µg/mL and 240.0µg/mL of Sofosbuvir.

A calibration curve with concentration versus peak areas was plotted by injecting the above-prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.5.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

$$LOD = 3.3\ \delta/S$$
$$LOQ = 10\ \delta/S$$

Where,

δ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

2.5.4. Method precision

The precision of the method was checked by repeated preparation(n=6) of 24.0µg/ml of Daclatasvir and 160.0µg/ml Sofosbuvir without changing the parameter of the proposed chromatographic method. And measured the peak areas and retention times.

2.5.5. Accuracy

The accuracy of the method was determined by calculating the recoveries of Daclatasvir and Sofosbuvir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Daclatasvir and Sofosbuvir.

2.5.6. Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min.

3. RESULTS AND DISCUSSIONS

Determination of Working Wavelength (λ max): 10 mg of the Daclatasvir and Sofosbuvir standard drug is taken in a 10 ml volumetric flask and dissolved in diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made up to the mark with the Water to give a concentration of 10 µg/ml. The above-prepared solution is scanned in UV between 200-400 nm using Water as blank. The λmax was found to be 260nm.

After several initial trails with mixtures of methanol, water, Acetonitrile and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (50:50). At flow rate was 1.0mL/ minute brought sharp peaks. The chromatogram was shown in Fig 3**.**

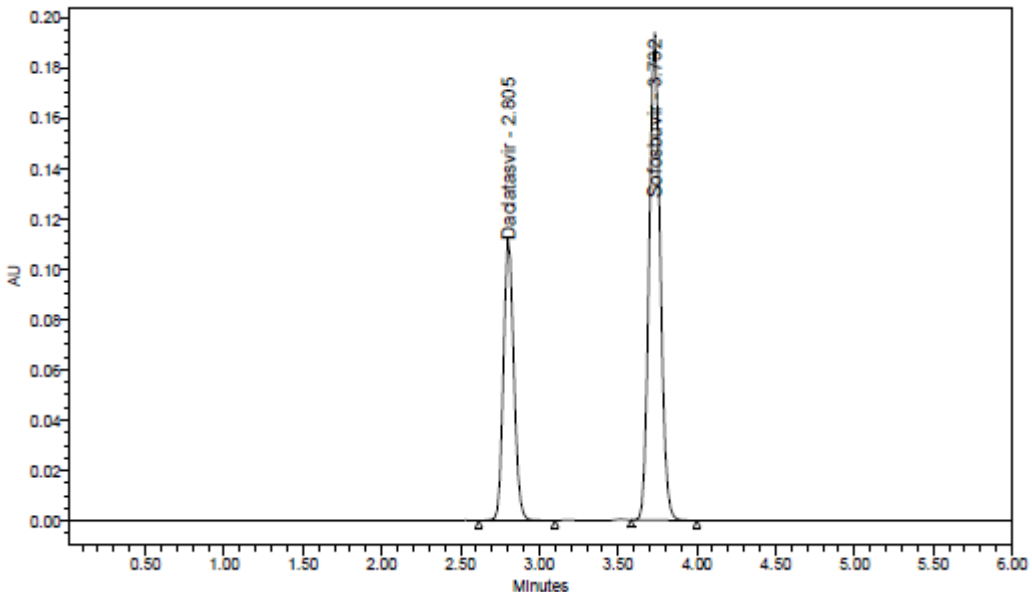


Fig. 3: Chromatogram of Daclatasvir and Sofosbuvir.

System suitability

The system suitability of the method was checked by repeated preparations for Sofosbuvir and Daclatasvir. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for the system, System suitability data of Sofosbuvir and Daclatasvir are shown in Table 1.

Table 1: System suitability data of Daclatasvir and Sofosbuvir.

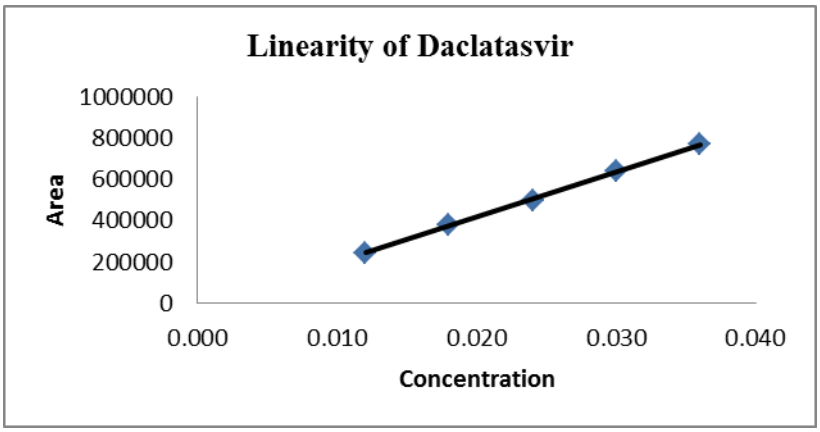
Parameter	Daclatasvir	Sofosbuvir	Acceptance criteria
Retention time	2.807	3.734	+-10
Theoretical plates	8438	12458	>3000
Tailing factor	1.11	1.08	<1.50
% RSD	0.38	0.31	<2.00

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 12.0 -36.0µg/ml for Daclatasvir and 80.0 -240.0µg/ml Sofosbuvir. From the primary stock solution 1ml, 1.5ml, 2ml, 2.5ml, 3 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 12.0 µg/mL, 18.0µg/mL, 24.0µg/mL, 30.0µg/mL and 36.0µg/mL of Daclatasvir and 80.0g/mL, 120.0µg/mL, 160.0µg/mL, 200.0µg/mL and 240.0µg/mL of Sofosbuvir in Table 2 and Table 3.

A linear relationship between peak areas versus concentrations was observed for Daclatasvir and Sofosbuvir in the range of 50% to 150% of nominal concentration. The correlation coefficient was 0.9997 and 1.000 for Daclatasvir and Sofosbuvir.

A



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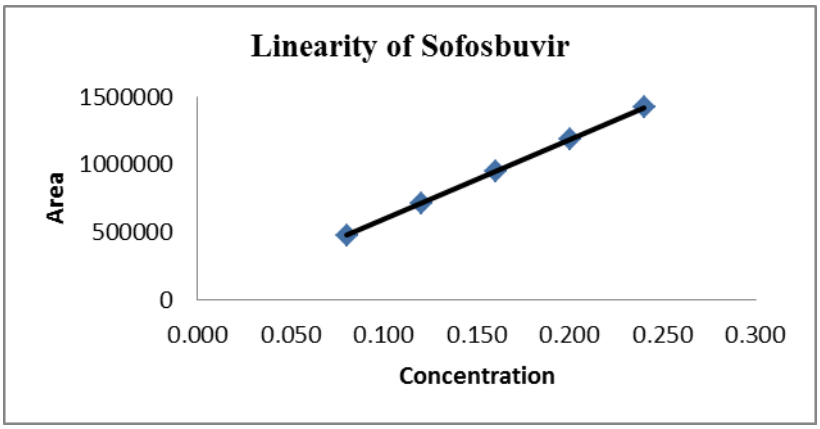

Fig. 4 Calibration curve: (A) Daclatasvir: (B) Sofosbuvir.

Table 2: Linearity data of Daclatasvir.

Level	Concentration (mg/mL)	Peak area
50%	0.012	243484
75%	0.018	379620
100%	0.024	499346
125%	0.030	643330
150%	0.036	770596
Correlation		0.9997

Table 3: Linearity data of Sofosbuvir.

Level	Concentration (mg/mL)	Peak area
50%	0.080	473932
75%	0.120	715863
100%	0.160	948361
125%	0.200	1190358
150%	0.240	1422045
Correlation		1.000

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 σ /S ……. (1)

LOQ =10 σ /S …….. (2)

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table 4: LOD and LOQ values Calculated from the calibration curve.

	Daclatasvir mg	Sofosbuvir mg
LOD	0.001	0.002
LOQ	0.003	0.005

Method precision (repeatability)

The precision of the method was checked by repeated preparation (n=6) of 24.0µg/ml of Daclatasvir and 160.0µg/ml Sofosbuvir without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times. The precision of the method (% RSD) was found to be <1% showing good repeatability. The values of percentage RSD for Daclatasvir and Sofosbuvir are shown in Table 5 and Table 6.

Table 5: Summary of peak areas for method precision of Daclatasvir.

	Retention time	Peak area	% Assay
1	2.808	506411	99.1
2	2.807	511193	98.9
3	2.805	505223	98.9
4	2.807	509294	99.5
5	2.805	505388	98.7
6	2.807	479589	98.9
Mean	2.807	502850	99.0
%RSD	**0.04**	**2.31**	**0.28**

Table 6: Summary of peak areas for method precision of Sofosbuvir.

Sample No	Retention time	Peak area	% Assay
1	3.735	955014	99.5
2	3.735	955683	99.8
3	3.733	956994	99.7
4	3.736	955986	99.9
5	3.733	950290	98.8
6	3.735	950886	99.1
Mean	3.735	954142	99.4
%RSD	**0.03**	**0.30**	**0.44**

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Daclatasvir and Sofosbuvir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Daclatasvir and Sofosbuvir. The percentage recovery results obtained are listed in Table 7 &8.

Table 7: Recovery data of Daclatasvir.

LEVEL	S.NO	%Recovery of Daclatasvir	Average
50	1	100.2	99.7%
	2	99.9	
	3	99.0	
100	1	99.1	99.0%
	2	98.9	
	3	98.9	
150	1	99.4	99.6%
	2	99.8	
	3	99.6	

Table 8: Recovery data of Sofosbuvir.

LEVEL	S.NO	%Recovery of Sofosbuvir	Average
50	1	99.6	99.6%
	2	99.0	
	3	100.2	
100	1	99.5	99.6%
	2	99.8	
	3	99.7	
150	1	99.7	99.4%
	2	99.6	
	3	99.0	

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table 9&10) the results of Robustness of the present method had shown that changes are not significant was found to be the method is Robust.

Table 9: Results of Daclatasvir.

parameter	Rt of Daclatasvir	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	3.848	9678	1.12
Increased flow rate (1.2ml/min)	2.349	7169	1.09
Wave Length 273nm	2.805	8379	1.12
277nm	2..811	8515	1.09

Table 10: Results of Sofosbuvir.

parameter	Rt of Sofosbuvir	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	4.636	14868	1.11
Increased flow rate (1.2ml/min)	3.120	10723	1.08
Wave Length 273nm	3.732	12545	1.08
277nm	3.736	12584	1.09

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table 11&12. The %RSD assay values between two analysts were calculated, this indicates the method was rugged.

Table 11: Ruggedness data for Daclatasvir.

		%Assay	%RSD
Analyst-1	**DACLATASVIR**	99.1	0.14%
Analyst-2		98.9	

Table 12: Ruggedness data for Sofosbuvir.

		%Assay	%RSD
Analyst-1	**SOFOSBUVIR**	99.5	0.21%
Analyst-2		99.8	

CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of DACLATASVIR and SOFOSBUVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost-effective and it can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

REFERENCES

1. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
2. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
3. http://www.ich.org/
4. https://en.wikipedia.org/wiki/Sofosbuvir
5. https://www.drugbank.ca/drugs/DB08934
6. https://en.wikipedia.org/wiki/Daclatasvir
7. https://www.drugbank.ca/drugs/DB09102
8. Stability indicating hplc method development and validation for determination of daclatasvir in pure and tablets dosage forms. hanaa saleh, gamal h. ragab, mohammed a. othman.
9. Method development and validation of ultraviolet-visible spectroscopic method for the estimation of hepatitis-c drugs - daclatasvir and sofosbuvir in active pharmaceutical ingredient form ashok chakravarthy v1*, sailaja bbv1, praveen kumar a2
10. Development and validation of new rp-hplc method for the determination of sofosbuvir in pure form p. mohan vikas*, dr. t. satyanarayana, d. vinod kumar, e. mounika, m. sri latha, r. anusha and y. sathish.